

Effect of insectary rearing on host preference and oviposition behavior of the fruit fly parasitoid *Diachasmimorpha longicaudata*

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Abstract

Diachasmimorpha longicaudata (Ashmead) has been produced in the laboratory for > 160 generations on the larvae of oriental fruit fly, *Bactrocera dorsalis* (Hendel), the propagation hosts raised routinely on a semi-synthetic wheat diet formulation. Choice tests using modified stinging units were conducted in the laboratory to investigate whether insectary rearing had altered the host seeking and oviposition behavior of female parasitoids. Results showed that fruit fly larvae that developed in papaya, *Carica papaya* L. var. 'solo', were less preferred for oviposition than fruit fly larvae that developed on wheat diet when both were exposed concurrently to naive *D. longicaudata* females (= females without prior oviposition experience). The substrates (pureed papaya or wheat diet) in which treatment larvae were exposed to parasitoids did not affect oviposition preference of gravid *D. longicaudata* for wheat diet-reared fruit fly larvae. Our study demonstrated the possibility that rearing in an insectary system may have modified the parasitization behavior of female *D. longicaudata*.

Introduction

Diachasmimorpha longicaudata (Ashmead) [= *Opius longicaudatus* Ashmead], a braconid parasitoid of tephritid fruit flies, was introduced into Hawaii between 1947 and 1952 subsequent to the discovery and establishment of the oriental fruit fly, *Bactrocera* (= *Dacus*) *dorsalis* (Hendel) (Zwaluwenberg, 1947; Clausen et al., 1965). This initial effort was undertaken primarily to deter spoilage of fruits and vegetables from fruit fly infestation and reduce the risks of fruit fly introductions to the continental United States through shipment of Hawaiian produce. Once very abundant in 1948, *D. longicaudata* accounted for only 30% of total fruit fly parasitization in 1979 and 1985 (Wong & Ramadan, 1987).

Diachasmimorpha longicaudata is an obligate endoparasitoid of *B. dorsalis* larvae. A female lays her eggs inside fruit fly larvae where they complete development (Greany et al., 1976). Adult parasitoids emerge from fruit fly pupae usually a few days after emergence

of adult flies from unparasitized pupae. When hosts are scarce, it is not uncommon for a female to oviposit more than 1 egg in a host larva (Lawrence et al., 1978). Nonetheless, *D. longicaudata* being solitary, only one individual from each pupa will complete development to adult.

From initial field collections of fruit fly pupae in the islands of Oahu, Maui, and Hawaii, a laboratory colony of *D. longicaudata* was started in 1981. Subsequently, a mass-rearing procedure was developed using larvae of *B. dorsalis* as propagation hosts (Wong & Ramadan, 1992). Currently, *D. longicaudata* is one of six species of fruit fly parasitoids reared in this laboratory which produces more than a million wasps each week (Wong & Ramadan, 1992).

D. longicaudata is commonly used in laboratory and field assays because it can be produced easily in large numbers (Wong & Ramadan, 1992). Notwithstanding, efforts to suppress resident populations of *B. dorsalis* in guavas through augmentative releases were unsuccessful on the island of Kauai, Hawaii

(M.F. Purcell, J.C. Herr, R.H. Messing & T.T.Y. Wong, unpubl.). Despite releases of 6–800 000 parasitoids during the first year of the test, parasitization of *B. dorsalis* in common guava, *Psidium guajava* L. hardly exceeded 6%. However, it is not known whether conditions during shipment of parasitized *B. dorsalis* pupae (Purcell et al., 1994) or errant behavior of adult parasitoids as they emerged and dispersed from release sites (Messing et al., 1994) may have affected the efficacy of *D. longicaudata*. Recently, in a study on interspecific competition among 3 parasitoids of *B. dorsalis*, we observed that females of *D. longicaudata* were less responsive in parasitizing fruit fly larvae infesting papayas (Bautista & Harris, unpubl.). Again, the reason why is not understood.

We were curious as to whether insectary rearing may have modified the parasitization behavior of *D. longicaudata*. Therefore, choice tests were undertaken in the laboratory to compare the host seeking and oviposition behavior of female parasitoids between fruit fly larvae that developed in *C. papaya* and those that developed on wheat diet.

Materials and methods

Rearing of fruit flies for assays. Approximately 1000 *B. dorsalis* eggs (18 to 24 h old), obtained from insectary-colonized fruit flies (Tanaka et al., 1969; Vargas, 1989), were inoculated in half-ripe whole *C. papaya* L. var. 'Solo' (weight range = 430–488 g per fruit) or seeded on 235 g wheat diet. Each of these rearing media was placed in 21 cm-diameter plastic containers with mesh screen lids. Five to 6 days later, following development of fruit fly larvae to 3rd instars (Wong & Ramadan, 1992), fruit fly larvae were teased out from deteriorated papaya with a pair of forceps then collected in a petri dish with tap water. In the case of the wheat diet, fruit fly larvae were screened from the wheat substrate with a mesh sieve (1 mm²) then emptied into another dish with tap water. Fruit fly larvae were taken at random from each petri dish for subsequent assays.

Experimental procedure. A modified stinging (= oviposition) unit developed and described by Wong & Ramadan (1992) was used in all the assays. A pair of stinging units, which contained fruit fly larvae, were exposed concurrently to 25 females of *D. longicaudata* (5 to 6-d-old after emergence) inside a cubical cage (26 × 26 × 26 cm). One of the stinging units contained

300 larvae reared in papaya while the other stinging unit contained 300 larvae reared on wheat diet. During the tests, adult parasitoids were fed with spun honey (Sioux Honey, Sioux City, IA) and agar (source of water).

Fruit fly larvae were not exposed naked to parasitoids but were mixed with wheat diet substrate before packing them in the stinging unit (Wong & Ramadan, 1992). In our tests, besides wheat diet, we likewise utilized pureed papaya as a substrate. Both substrates were used merely for purposes of confirming whether odor stimuli other than those that may be associated with the treatment larvae would influence the choice made by the female parasitoids. Wheat millfeed (Armstrong, B.C. Canada), a bulking material routinely used in *B. dorsalis* larval diet formulation, was mixed with pureed papaya or wheat diet substrate in equal parts (by weight) to make mixture drier. Otherwise, female parasitoids would shy away from the wet surface of the stinging unit (Wong & Ramadan, 1992).

All assays were conducted under laboratory conditions with mean ambient low and high temperatures of 23 ± 0.3 °C and 24 ± 0.2 °C, respectively, relative humidity of $61.5 \pm 0.8\%$, and L10:D14 photoperiod. Each test was replicated 12 times. Fresh cohorts of naive *D. longicaudata* females (= no prior oviposition experience) were used in each replication.

Test 1. Fruit fly larvae reared in papaya (*Pa*) or wheat diet (*Wd*) were exposed to parasitoids in pureed papaya substrate (*Pus*) or wheat diet substrate (*Wds*), respectively. Treatments were subsequently referred to as (*Pa-Pus*)₁ and (*Wd-Wds*)₁, respectively. The numerical subscript in each treatment indicates the test number.

One half hour after initial exposure of (*Pa-Pus*)₁ and (*Wd-Wds*)₁, and at hourly intervals for a total exposure time of 6 h, the number of parasitoids on each stinging unit that exhibited typical host seeking behavior, i.e., intense antennation on surface of stinging unit, extension of ovipositor, and insertion of ovipositor accompanied by pumping movements, were recorded. The number of females observed on each stinging unit per unit time was expressed in percentage based on the original number of females assayed in each cage.

At the end of the 6 h period (total exposure time), the pair of stinging units were retrieved from the cage, then 30 larvae were sampled at random from each stinging unit. Fruit fly larvae were dissected individually under a stereo microscope for presence of parasitoid eggs. Percentage parasitization (pooled counts of para-

sitized fruit fly larvae which contained 1 and 2 or more parasitoid eggs) was computed by dividing the number parasitized by total number dissected.

The remainder of fruit fly larvae from each stinging unit was placed in separate holding cups (11 cm-diameter) provisioned with vermiculite (W. R. Grace, Cambridge, MA) for fruit fly pupation. After 9–10 d fruit fly pupae were sifted with a mesh screen (1 mm²). A cohort of 200 pupae was sampled and then placed in emergence cups with mesh-screen covers until eclosion of parasitoids. Pooled counts of emerged parasitoids and dead parasitoid cadavers in unclosed pupae were recorded. Percent yields of parasitoid progeny in each treatment was expressed as a ratio of total number of parasitoids that developed per 200 pupae sampled.

Test 2. The same variables were generated according to procedure described in preceding test except that fruit fly larvae reared in *Pa* or *Wd* were both exposed to parasitoids in *-Wds* substrate only. Treatments were subsequently referred to as (*Pa-Wds*)₂ and (*Wd-Wds*)₂.

Test 3. Fruit fly larvae reared in *Pa* and *Wd* were both exposed to parasitoids in *-Pus* substrate only. As in Test 1, similar procedure was followed in the processing of samples and data collection. Treatments were subsequently referred to as (*Pa-Pus*)₃ and (*Wd-Pus*)₃.

Data analysis. Data on proportion of responding *D. longicaudata* females, rate of parasitization, and percentage parasitoid progeny yield were compared between paired treatments by Student's *t*-test at *P* = 0.05. Percentages were transformed to arcsine $\sqrt{\text{proportion}}$ before analyses. Untransformed means (\pm SEM) are used in presentation of results.

Results

Test 1. The overall mean percentage of female parasitoids that responded to fruit fly larvae following a total exposure time of 6 h was 35% higher in (*Pa-Pus*)₁ than those in (*Wd-Wds*)₁ (*t* = 4.86, *P* < 0.0001). Commencing at 0.5 h (*t* = 4.40, *P* < 0.0001) and at 1 h (*t* = 5.26, *P* < 0.0001), 2 h (*t* = 4.35, *P* = 0.0003), 3 h (*t* = 5.20, *P* < 0.0001), 4 h (*t* = 3.86, *P* = 0.0008), 5 h (*t* = 4.38, *P* = 0.0002), and 6 h (*t* = 3.96, *P* = 0.0006) thereafter, significantly more female parasitoids (range = 25 to > 40%) were consistently observed on (*Pa-Pus*)₁ than (*Wd-Wds*)₁ (Figure 1).

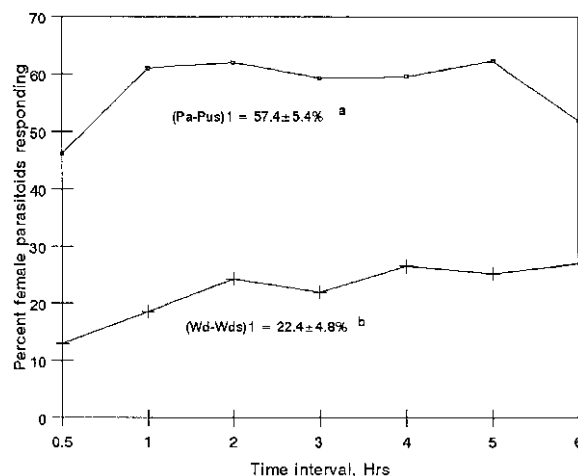


Figure 1. TEST 1: Proportion of *D. longicaudata* females that responded to papaya (*Pa*) or wheat diet-reared (*Wd*) *B. dorsalis* larvae when both were exposed concurrently to parasitoids in papaya puree (*-Pus*) or wheat diet (*-Wds*) substrate, respectively. Overall means between treatments with same letter are not significantly different by Student's *t*-test, *P* > 0.05.

Although mean percent parasitization of fruit fly larvae in (*Wd-Wds*)₁ was ca. 20% higher than in (*Pa-Pus*)₁, difference between treatments was not statistically significant (*P* = 0.09) (Table 1). Likewise, mean yield was comparable between treatments (*P* = 0.078) (Table 1) although ca. 12% more parasitoid progeny were obtained in (*Wd-Wds*)₁ than in (*Pa-Pus*)₁.

Test 2. From initial time of fruit fly larval exposure (0.5 h) until 2 h later, female parasitoids did not show any particular preference for treatment larvae in either of the two stinging units (Figure 2). However, more and more females were lured to (*Wd-Wds*)₂ than (*Pa-Pus*)₂ as exposure time lengthened until the 5th h (*t* = -4.0, *P* = 0.0006) and 6th h (*t* = -3.21, *P* = 0.004). Overall, the mean proportion of female parasitoids that responded to fruit fly larvae in (*Wd-Wds*)₂ was significantly higher than those in (*Pa-Pus*)₂ (*t* = -2.22, *P* = 0.038). Moreover, fruit fly larvae in (*Wd-Wds*)₂ were intensely oviposited by female parasitoids than those in (*Pa-Wds*)₂ thus, percentage larval parasitization was 35% higher in (*Wd-Wds*)₂ than parasitization obtained in (*Pa-Wds*)₂ (Table 1). Significant differences in progeny yield between (*Wd-Wds*)₂ and (*Pa-Wds*)₂ were likewise consistent with dissection data.

Test 3. Relatively more female parasitoids (range = 3–11%) were observed on (*Wd-Pus*)₃ than those on

Table 1. Mean percent larval parasitization of *B. dorsalis* and parasitoid progeny yield compared between fruit fly larvae that developed in papaya or wheat diet then exposed concurrently to *D. longicaudata* in a pair of stinging units. Treatment means were compared in each test by Student's *t*-test, $P = 0.05$

Test No.	Treatments ^a	Percent (\pm SEM) parasitization of fruit fly larvae	Percent (\pm SEM) yield of parasitoid progeny
1	(Pa-Pus) ₁	29.9 \pm 7.8	13.5 \pm 4.4
	(Wd-Wds) ₁	50.9 \pm 6.9	25.1 \pm 4.5
	<i>t</i>	-1.99	-1.85
	P	0.09	0.078
2	(Pa-Wds) ₂	9.17 \pm 4.6	10.9 \pm 3.3
	(Wd-Wds) ₂	44.2 \pm 5.8	25.8 \pm 3.1
	<i>t</i>	-4.72	-3.29
	P	0.003	0.004
3	(Pa-Pus) ₃	37.5 \pm 8.3	20.9 \pm 3.5
	(Wd-Pus) ₃	76.7 \pm 4.9	32.2 \pm 3.8
	<i>t</i>	-4.05	-2.16
	P	0.007	0.042

^a Abbreviations *Pa* and *Wd* refer to papaya fruit or wheat diet, respectively, the culture media in which fruit fly larvae developed. The suffixes *-Pus* and *-Wds* refer to papaya puree or wheat diet, respectively, the substrates in which treatment larvae were mixed with and exposed to parasitoids in stinging units. Numerical subscripts indicate test numbers.

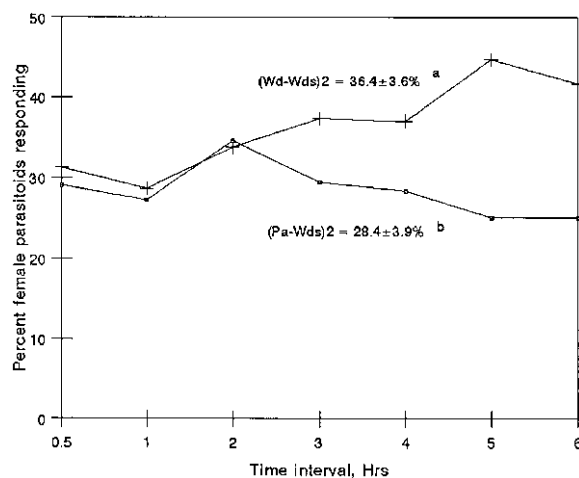


Figure 2. TEST 2: Proportion of *D. longicaudata* females that responded to papaya (*Pa*) or wheat diet-reared (*Wd*) *B. dorsalis* larvae when both were exposed concurrently to parasitoids in wheat diet (*-Wds*) substrate only. Overall means between treatments with same letter are not significantly different by Student's *t*-test, $P > 0.05$.

(*Pa-Pus*)₃ (Figure 3). However, variation in the number of responding females was not sufficiently large to be

significantly different between treatments ($P = 0.115$). Nonetheless, (*Wd-Pus*)₃ had significantly more larvae (39%) parasitized by *D. longicaudata* than host larvae dissected in (*Pa-Pus*)₃ (Table 1). Moreover, mean parasitoid progeny that developed in (*Wd-Pus*)₃ was significantly higher than that obtained in (*Pa-Pus*)₃.

Discussion

Insectary-colonized *D. longicaudata* showed a diminished preference for *B. dorsalis* larvae other than those that developed on semi-synthetic wheat diet formulation. Our results indicated that routine propagation of *D. longicaudata* on wheat diet-reared fruit fly larvae had modified parasitization behavior of female parasitoids.

Regardless of the substrates (pureed papaya or wheat diet) with which larval hosts were exposed to *D. longicaudata* (tests 2 and 3), more female parasitoids responded to the stinging unit which contained wheat diet rather than papaya-reared fruit fly larvae. Moreover, the preference of *D. longicaudata* for wheat

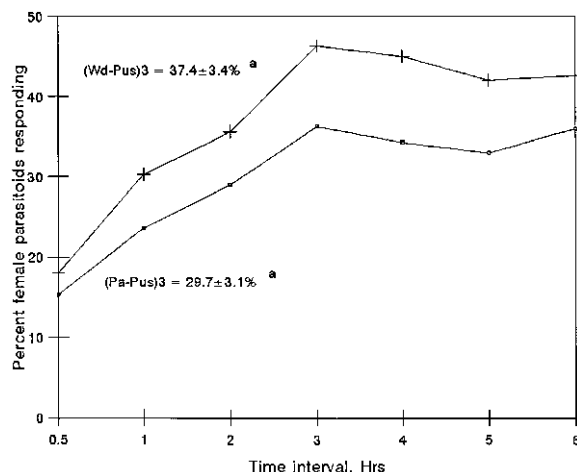


Figure 3. TEST 3: Proportion of *D. longicaudata* females that responded to papaya (*Pa*) or wheat diet-reared (*Wd*) *B. dorsalis* larvae when both were exposed concurrently to parasitoids in papaya puree (*-Pus*) substrate only. Overall means between treatments with same letter are not significantly different by Student's *t*-test, $P > 0.05$.

diet-reared fruit fly larvae was consistent with heavy parasitization of *B. dorsalis* larvae and higher parasitoid progeny yields.

Some discrepancy in our results, however, was obtained in test 1. The host seeking response exhibited by gravid *D. longicaudata* was more intense in (*Pa-Pus*)₁ rather than in (*Wd-Wds*)₁. Surprisingly, said female behavior did not result in significantly higher percentage of parasitized fruit fly larvae and parasitoid progeny yield. In fact, relatively more host larvae were parasitized or parasitoid yield was slightly better in (*Wd-Wds*)₁ than those in (*Pa-Pus*)₁. Variation between treatments may not be statistically significant, but our findings confirmed previous observations that female *D. longicaudata* has a predilection for natural fruit odor stimuli, in this case, papaya puree used as substrate in the exposure of papaya-reared fruit fly larvae (Messing & Jang, 1992; Greany et al., 1977a; Purcell et al., 1994). And, that the host seeking response elicited by female parasitoids in (*Pa-Pus*)₁ did not necessarily indicate successful oviposition. Apparently, rather than laying eggs, female parasitoids spent considerable time probing and searching for more acceptable hosts (Lawrence, 1978), most likely, with the aid of sense organs associated with their ovipositors. These organs serve as receptors for oviposition inducing stimuli that emanate from the hosts (Greany et al., 1977b).

We could not ascertain the mechanism that may have influenced *D. longicaudata*'s preference for wheat diet-reared fruit fly larvae although sever-

al investigators have suggested that the behavioral response of some parasitoids at the adult stage may be modified before or during emergence through a chemical legacy by parasitoid immatures which developed on artificially produced insect hosts (Vet, 1983, 1985a; Corbet, 1985; Herard et al., 1988). Likewise, our data could not substantiate if prior experience of *D. longicaudata* [as preimago] on wheat diet-reared *B. dorsalis* larvae and subsequent female behavior may have been selected for by their progenitors during many generations of adaptation to laboratory conditions. Nonetheless, our findings can not preclude these possibilities considering that *D. longicaudata* has been produced routinely on wheat diet-reared *B. dorsalis* for > 160 successive generations.

The suboptimum performance of *D. longicaudata* when assayed against *B. dorsalis* in previous field or laboratory tests may have been a product of a change in host seeking and parasitization behavior of female parasitoids. We do not know if this behavioral switch is permanent or transitory (= plasticity of trait) in nature. Nevertheless, within the context of fruit fly pest management and, considering that host preference of *D. longicaudata* for fruit flies varies with different fruit varieties (Leyva et al., 1991), this phenomenon may have detrimental consequences in parasitoid release programmes.

Large scale production of insects at minimal costs, space, and man-hours is the paramount goal of any insect mass-rearing program (Finney & Fisher, 1964; Knipling, 1966). However, insect numbers must not compromise the quality of insects produced, particularly their behavioral traits (Huettel, 1976). We can not overlook the importance of basic research concerning the behavior of laboratory-reared insect populations. Based on our findings, we suggest incorporating procedures in insectary rearing which would enable researchers to assess field quality and behavior of *D. longicaudata* and other insectary-propagated fruit fly parasitoids.

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